SUBSTITUTE FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
10848-021US1

U S. APPLICATION NO. (If Known, see 37 CFR 1.5)
10/069840

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/02757	12 August 2000	26 August 1999

TITLE OF INVENTION
METHOD FOR DETECTING AND QUANTIFYING FIRST BIOPOLYMERS THAT ARE LOCATED IN A LIQUID

APPLICANT(S) FOR DO/EO/US
Wolf Bertling, Jurgen Schulein and Jorg Hassmann

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern other documents or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    ☒ PCT International Search Report (4 pages) with 5 references attached.
    ☐
    ☐
    ☐
    ☐

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February 26, 2002
Date of Deposit

Signature

Vince Defante
Typed Name of Person Signing

U.S. APPLICATION NO. (IF KNOWN) 10/069840	INTERNATIONAL APPLICATION NO. PCT/DE00/02757	ATTORNEY'S DOCKET NUMBER 10848-021US1

17. ☒ The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)- (5)):

	CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00
Surcharge of **$130** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$0.00

Claims	Number Filed	Number Extra	Rate		
Total Claims	13 - 20 =		x **$18**	$0.00	
Independent Claims	1 - 3 =		x **$84**	$0.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ **$280**	$0.00	
			TOTAL OF ABOVE CALCULATIONS =	$890.00	
☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$445.00	
			SUBTOTAL =	$445.00	
Processing fee of **$130** for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f))				$0.00	
			TOTAL NATIONAL FEE =	$445.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$0.00	
			TOTAL FEES ENCLOSED =	$445.00	
			Amount to be refunded:		$
			Charged:		$

a. ☒ A check in the amount of $445.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 06-1050 in the amount of $0.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO.

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SIGNATURE : M. Angela Parsons

M. Angela Parsons, Ph.D.
NAME

44,282
REGISTRATION NUMBER

#4

Attorney's Docket No.: 10848-021US1 / 422073GA-go

IN THE UNITED STATES RECEIVING OFFICE

Applicant : Wolf Bertling et al. Art Unit : Unknown
Serial No. : 10/069,840 Examiner : Unknown
Filed : February 26, 2002
Title : METHOD FOR DETECTING AND QUANTIFYING FIRST BIOPOLYMERS THAT ARE LOCATED IN A LIQUID

BOX PCT
Commissioner for Patents
Washington, D.C. 20231

VERIFIED STATEMENT UNDER 37 CFR §1.821(f)

I, Judith A. Wasilkus, declare that I personally prepared the paper and the computer-readable copy of the Sequence Listing filed herewith for the above-identified application and that the content of both is the same.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of The United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: June 26, 2002

Judith A. Wasilkus
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60090251.doc

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June 26, 2002
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Signature

Vince Defante
Typed or Printed Name of Person Signing Certificate

 #4

Attorney's Docket No.: 10848-021US1 / 422073GA-go

IN THE UNITED STATES RECEIVING OFFICE

Applicant : Wolf Bertling et al.
Serial No. : 10/069,840
Filed : February 26, 2002
Title : METHOD FOR DETECTING AND QUANTIFYING FIRST BIOPOLYMERS THAT ARE LOCATED IN A LIQUID

BOX PCT
Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202

RESPONSE AND AMENDMENT

In response to the Notification of Missing Requirements dated April 26, 2002 (copy enclosed), Applicants, as a Small Entity, submit herewith a paper copy of the Sequence Listing as required under 37 CFR §1.821-1.825, and the Sequence Listing in computer readable form as required by 37 CFR §1.824. In addition, Applicants submit a Verified Statement indicating that the contents of the paper copy and computer readable form are identical as required under 37 CFR §1.821(f).

Applicants respectfully request entry of the paper copy and computer readable form of the Sequence Listing filed herewith. Furthermore, Applicants request entry of the following amendments.

In the Specification:

Please replace the original Sequence Listing with the Sequence Listing filed herewith.

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[redacted]

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 CFR §1.821-1.825. The amendments to the specification merely insert the paper copy of the Sequence Listing into the application. No new matter has been added. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: June 26, 2002

M. Angela Parsons

M. Angela Parsons, Ph.D.
Reg. No. 44,282

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60092014.doc

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The original Sequence Listing was replaced with the Sequence Listing filed herewith.

SEQUENCE LISTING

<110> Bertling, Wolf
Schulein, Jurgen
Hassmann, Jorg

<120> Method for Detecting and Quantifying
First Biopolymers That Are Located in a Liquid

<130> 10848-021US1

<140> US 10/069,840
<141> 2002-02-26

<150> PCT/DE00/02757
<151> 2000-08-12

<150> DE 19940647.2
<151> 1999-08-26

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growth hormone
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Wolf Bertling et al. Art Unit : Unknown
Serial No. : Examiner : Unknown
Filed :
Title : METHOD FOR DETECTING AND QUANTIFYING FIRST BIOPOLYMERS THAT ARE LOCATED IN A LIQUID

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the Specification:

Please add the following paragraph to the application after the title:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a National Stage application under 35 U.S.C. §371 and claims benefit under 35 U.S.C. §119(a) of International Application No. PCT/DE00/02757 having an International Filing Date of August 12, 2000, which claims benefit of DE 199 40 647.2 filed on August 26, 1999.--

Please delete the paragraph on page 2, lines 13-15.

In the Claims:

Please amend claims 1-4 and 7-13 as indicated below. Please cancel claim 14. A full set of pending claims is shown for convenience.

1. (Amended) A method for detecting and quantifying first biopolymers (1) that are located in a liquid, where second biopolymers (2) which have a specific affinity to the first

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Vince Defante
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biopolymers (1) to be detected are bonded to the surface of a first electrode (E1), and where the first and at least one second electrode (E2) are in contact with the liquid, said method having the following steps:

contacting the liquid with the first electrode (E1),

applying a voltage and/or current across the first electrode (E1) and the second electrode (E2), and

measuring a direct change in the voltage and/or current caused by addition of the first biopolymers (1) onto the second biopolymers (2).

2. (Amended) A method as claimed in claim 1, where a direct-voltage signal is measured.

3. (Amended) A method as claimed in claim 2, where the measuring is a cyclovoltammetric measuring.

4. (Amended) A method as claimed in claim 1, further comprising plotting the measured current or the measured voltage against time and integrating at least one peak.

5. A method as claimed in claim 1, where an alternating-current signal is measured phase-sensitively.

6. A method as claimed in claim 5, where the alternating-current signal is superimposed on a cyclic direct-current signal.

7. (Amended) A method as claimed in claim 1, further comprising measuring impedance by measuring voltammetric signals at varying frequency.

8. (Amended) A method as claimed in claim 1, further comprising increasing the concentration of the first biopolymers (1) at the surface of the first electrode (E1) by application of a voltage and/or current prior to contacting the liquid with the first electrode (E1).

9. (Amended) A method as claimed in claim 8, where polarity is reversed cyclically.

10. (Amended) A method as claimed in claim 8, where the measuring is performed in a defined measurement solution.

11. (Amended) A method as claimed in claim 1, where a first end of the second biopolymer (2) is bonded to the surface of the first electrode (E1) via a covalent bond or via a linker.

12. (Amended) A method as claimed in claim 11, where the first electrode (E1) is made of plastic, ceramic, glass or metal.

13. (Amended) A method as claimed in claim 1, where the first biopolymer (1) is a single-stranded DNA or RNA which is complementary to the second biopolymer (2).

In the Abstract:

Please add the attached Abstract to the application after the claims.

REMARKS

Applicants respectfully request entry of the amendments and remarks submitted herein. Claims 1-4 and 7-13 have been amended, and claim 14 has been canceled. Claims 1-13 are currently pending. Attached is a marked-up version of the changes being made by the current amendments. Examination of the pending application is respectfully requested.

In addition, Applicants have amended the specification to include a paragraph describing related applications and to claim the benefit of priority to such applications. Applicants also have amended the specification to remove the paragraph on page 2 that refers to claim numbers, and to add an Abstract. The attached Abstract is the English language Abstract that was published with the PCT application. Therefore, Applicants submit that there is no new matter introduced by these amendments.

CONCLUSION

Applicants ask that claims 1-13 be examined. The enclosed filing fee takes into account the claims pending following entry of this Preliminary Amendment. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: February 26, 2002

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60073384 doc

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Information on Cross-Related applications has been added to the specification after the title.

The paragraph on page 2, lines 13-15 has been deleted.

In the Claims:

Claims 1-4 and 7-13 have been amended as follows:

1. (Amended) A method for detecting and quantifying first biopolymers (1) that are located in a liquid, where second biopolymers (2) which have a specific affinity to the first biopolymers (1) to be detected are bonded to the surface of a first electrode (E1), and where the first and at least one second electrode (E2) are in contact with the liquid, said method having the following steps:

contacting the liquid with the first electrode (E1),

[a) application of] applying a voltage and/or current across the first electrode (E1) and the second electrode (E2), and

[b) measurement of] measuring a direct change in the voltage and/or current caused by addition of the first biopolymers (1) onto the second biopolymers (2).

2. (Amended) A method as claimed in claim 1, where [in step b),] a direct-voltage signal is measured.

3. (Amended) A method as claimed in claim 2, where the [measurement] measuring is [carried out as] a cyclovoltammetric measuring [measurement].

4. (Amended) A method as claimed in claim 1, [2 or 3, where, for the detection and quantification of the first biopolymer (1),] further comprising plotting the measured current or the measured voltage [is plotted] against time and integrating [integrated over] at least one peak.

7. (Amended) A method as claimed in claim 1, [where the] further comprising measuring impedance[is measured] by measuring [the] voltammetric signals at varying frequency.

8. (Amended) A method as claimed in claim 1, [one of the preceding claims, where, before step a),] further comprising increasing the concentration of the first biopolymers (1) [are increased in concentration] at the surface of the first electrode (E1) by application of a voltage and/or current prior to contacting the liquid with the first electrode (E1).

9. (Amended) A method as claimed in claim 8, where [the] polarity is reversed cyclically.

10. (Amended) A method as claimed in claim 8 [or 9], where the measuring is performed in [first electrode (E1) with the first biomolecules (1) increased in concentration at it is removed from the liquid and, for the measurement, brought into contact with] a defined measurement solution.

11. (Amended) A method as claimed in claim 1 [one of the preceding claims], where a first end of the second biopolymer (2) is bonded [by means of one end] to the surface of the first electrode (E1) via a covalent bond or via a linker.

12. (Amended) A method as claimed in claim 11, where the first electrode (E1) is made of [one of the following materials:] plastic, ceramic, glass or metal.

13. (Amended) A method as claimed in claim 1 [one of the preceding claims], where the first biopolymer (1) is a single-stranded DNA or RNA which is complementary to the second biopolymer (2).

Claim 14 has been canceled.

In the Abstract:

The Abstract on the attached page has been added to the application.

ABSTRACT OF THE DISCLOSURE

The invention realtes to a method for detecting and quantifying first biopolymers (1) that are located in a liquid. Second biopolymers (2) having a specific affinity towards the first biopolymers (1) to be detected are bound to the surface of a first electrode (E1). The first and at least one second electrode are dipped into the liquid. The inventive method comprises the following steps: a) applying a changeable voltage and/or a changeable current over the first (E1) and second electrode (E2) and b) measuring the direct change of voltage and/or current, whereby said change is the result of the accumulation of the first biomolecules (1) to the second biomolecules (2).

3/pats

Method for detecting and quantifying first biopolymers that are located in a liquid

The invention relates to a method for detecting and quantifying first biopolymers that are located in a liquid.

It is known from the prior art that polynucleotide sequences, such as DNA, can be detected by voltammetric methods. To this end, it is proposed in US 5,312,572 to add redox-active molecules to the solution. In the case of hybridization, these molecules bind to the double-stranded molecule formed from the polynucleotide sequences. The redox-active molecule causes a measurable redox signal. A similar method is also disclosed in US 5,871,918.

WO 96/01836 discloses a chip for detecting polynucleotide sequences. A multiplicity of miniaturized reaction cavities are provided on the chip. A particular polynucleotide sequence is bound in each of the reaction cavities. On immersion of the chip into a solution containing the polynucleotide sequence to be detected, hybridization occurs with one of the particular polynucleotide sequences. The hybridization is detected by means of fluorescence.

WO 95/12808 also relates to a detection method using a chip. In this method, a voltage is applied across the chip in the manner of an electrode. Charged polynucleotide sequences that are located in the solution can thus be increased in concentration at the surface of the chip or at the miniaturized reaction vessels provided therein.

The prior-art methods are complex. They require the addition of particular redox-active molecules or the presence of chips which are complex to manufacture.

Precise quantification of the biopolymers to be detected is not possible using the known methods. Optical detection is usually necessary. This increases the apparatus complexity.

The object of the invention is to overcome the disadvantages of the prior art. In particular, the object is to indicate a method for detecting and quantifying biopolymers that are located in a liquid which can be carried out in the simplest and most inexpensive manner possible.

This object is achieved by the features of claim 1. Advantageous embodiments arise from the features of claims 2-14.

The invention proposes a method for detecting and quantifying first biopolymers that are located in a liquid in which second biopolymers which have a specific affinity to the first biopolymers to be detected are bound to the surface of a first electrode, and in which the first and at least one second electrode are in contact with the liquid, having the following steps:

a) application of a voltage and/or a current across the first and second electrodes, and

b) measurement of a direct change in the voltage and/or the current which is caused by the addition of the first biomolecules onto the second biomolecules.

The method proposed can be carried out simply and quickly. The change in the voltage and/or current is measured directly, i.e. it is not necessary to provide particular redox-active molecules. In particular, quantification of the first biopolymers that are

present in the liquid and are to be detected is also possible in a simple manner.

The applied voltage or current may be changeable, i.e. it may be alternating current. In step b, a direct-current signal can be measured, the measurement advantageously being carried out as a cyclovoltammetric measurement. In this case, the redox processes of the first biopolymers added onto the second biopolymers and Faraday electron transfer through the surface of the first electrode at a prespecified voltage or in a pre-specified voltage range are utilized.

For the detection and quantification of the first biopolymer, the measured current or the measured voltage is advantageously plotted against time and integrated at least over one peak. The integration is advantageously carried out with subtraction of the background. The amount of charge transferred through the adding-on of the first biopolymers can be determined from the value arising in the integration. This can be used to conclude the number of first biopolymers added on. Calibration is possible. The concentration of the first biopolymers to be detected in the liquid can be determined. The proposed method is particularly simple since here - as in conventional cyclic voltammetry - a linear voltage ramp is passed through cyclically only between the first electrode and a reference electrode. It is not necessary to employ redox-active molecules for charge transport.

The current flow can be measured via a third electrode or a counterelectrode. When the measured current or measured voltage is plotted against time, characteristic peaks can be observed which can be assigned to addition or adsorption and to desorption of the first biopolymers.

A further embodiment comprises measuring an alternating-current signal phase-sensitively. The addition of the first biopolymers onto the second biopolymers which are bound terminally to the first electrode causes a change in the double layer capacitance caused by the addition and repulsion of the charged biopolymers. The phase-sensitive measurement proposed enables the capacitive proportion of the alternating-current signal to be determined. Knowledge of the capacitive proportion allows conclusions to be drawn on the concentration of the first biopolymer to be detected in the liquid.

In this connection, it is possible to superimpose the alternating-current signal on a cyclic direct-current signal. According to a further embodiment of the method, the impendance is measured by measuring the voltammetric signals at varying frequency. This enables direct quantification of the degree of coverage of the surface of the first electrode by first biopolymers to be detected.

It is advantageous, before step a, to increase the concentration of the first biopolymers at the surface of the first electrode by applying a voltage and/or current. The increasing concentration is particularly effective if the polarity is reversed cyclically. Molecules which are not complementary to the second biopolymers are thereby repelled by the surface.

According to a further design feature, it is proposed that the first electrode with the first biomolecules increased in concentration at it is removed from the liquid and, optionally after carrying out a washing step, brought into contact with a defined measurement solution for the measurement. This enables interfering influences due to any further electrochemically active species that may be present in the liquid to be

substantially suppressed. Pre-measurement purification takes place to a certain extent.

The second biopolymer is advantageously bonded by means of one end to the surface of the first electrode via a covalent bond or via a linker. It is of course also possible for spacer molecules to be inserted in between for the bonding. The first electrode is advantageously made of one of the following materials: plastic, ceramic, glass or metal. In particular, polycarbonates and gold have proven particularly advantageous as electrode materials.

The terms first biopolymer and second biopolymer here are taken to mean, in particular, proteins, peptides, DNA, RNA and the like. The first biopolymer can be, in particular, a single-stranded DNA or RNA which is complementary to the second biopolymer. In step b, the change in voltage and/or current caused by the hybridization of the above-mentioned biopolymers is thus preferably measured.

The method is explained in greater detail with reference to working examples, in which

Fig. 1 shows a cyclic voltammogram, and

Fig. 2 shows the change in alternating current plotted against the frequency, and

Fig. 3 shows a diagrammatic view of a measurement set-up.

Example 1: Direct-voltage voltammetric measurement

Part of a DNA sequence (HGH1) which encodes for human growth hormone is bonded at its 5' end to a first electrode E1 or working electrode made from poly-

carbonate/carbon fibers. The first electrode E1 dips into a solution containing 5 fM/µl of a complementary DNA sequence (HGH1 comp.), 80 mM TBE buffer and 100 mM NaCl conductive salt.

A voltage is increased in a linear manner from 0 to 1.3 V at a rate of change of 10 mV/s, then moved back to -1.3 V and finally up to 0 V again. The cyclic voltammogram measured during this operation is shown in Fig. 1. Two peaks are evident. In the anodic potential region, a first peak P1 occurs at U = 749.5 mV against Ag/AgCl, and in the cathodic potential region, a second peak P2 arises at U = -864.3 mV against Ag/AgCl. These peaks only occur if second biopolymers which are complementary to first biopolymers bound to the surface of the electrode are present in the solution. The peaks P1 and P2 are exclusively attributable to redox processes caused, in the present example, by hybridization of HGH1 with HGH1 comp. The first peak P1 located in the anodic region is the consequence of a redox process caused by adsorption or addition of HGH1 onto HGH1 comp. The second peak P2 which can be observed in the cathodic region can be associated with desorption of the hybridized nucleic acids. Integration of the first peak P1 over time corresponds to the amount of charge transported by the electrode surface. This enables conclusions to be drawn on the degree of hybridization on the surface of the first electrode.

Example 2: Alternating-current voltammetric measurement

The same electrode and solution are used as in Example 1.

A direct-voltage scan from 0 V to 1 V against Ag/AgCl is set up, and an alternating voltage of 250 Hz is superimposed on this direct voltage. The alternating current is measured in a phase shift of 90° to the

alternating voltage. If second biopolymers which are complementary to the first biopolymers that are located in the solution and to be detected are present on the first electrode, the peak shown in Fig. 2 arises at 250 mV. This peak is caused by a change in capacitance due to transport of negative charges to the surface of the first electrode during hybridization. The change in the double-layer capacitance results in a capacitive current flow, which can be detected by phase-sensitive measurement of the alternating-current fraction. The upper curve shows the alternating-current signal in the case of a first electrode with no biopolymer on the surface. No peak can be observed here.

Fig. 3 shows the measurement set-up in diagrammatic form. A first electrode E1, a second electrode E2 and a third electrode E3 dip into a liquid. The first electrode E1 is the working electrode. Second biopolymers, for example DNA, are covalently bonded thereto by means of a linker. First biopolymers (1) which are complementary to the second biopolymers (2) are located in the liquid. The second electrode (E2) serves as counterelectrode, the third electrode (E3) as reference electrode.

The following sequence listing shows the sequences of HGH1 and HGH1 comp.

Patent Claims

1. A method for detecting and quantifying first biopolymers (1) that are located in a liquid, where second biopolymers (2) which have a specific affinity to the first biopolymers (1) to be detected are bonded to the surface of a first electrode (E1), and where the first and at least one second electrode (E2) are in contact with the liquid, having the following steps:

a) application of a voltage and/or current across the first electrode (E1) and the second electrode (E2), and

b) measurement of a direct change in the voltage and/or current caused by addition of the first biopolymers (1) onto the second biopolymers (2).

2. A method as claimed in claim 1, where in step b), a direct-voltage signal is measured.

3. A method as claimed in claim 2, where the measurement is carried out as a cyclovoltammetric measurement.

4. A method as claimed in claim 2 or 3, where, for the detection and quantification of the first biopolymer (1), the measured current or the measured voltage is plotted against time and integrated over at least one peak.

5. A method as claimed in claim 1, where an alternating-current signal is measured phase-sensitively.

6. A method as claimed in claim 5, where the alternating-current signal is superimposed on a cyclic direct-current signal.

7. A method as claimed in claim 1, where the impedance is measured by measuring the voltammetric signals at varying frequency.

8. A method as claimed in one of the preceding claims, where, before step a), the first biopolymers (1) are increased in concentration at the surface of the first electrode (E1) by application of a voltage and/or current.

9. A method as claimed in claim 8, where the polarity is reversed cyclically.

10. A method as claimed in claim 8 or 9, where the first electrode (E1) with the first biomolecules (1) increased in concentration at it is removed from the liquid and, for the measurement, brought into contact with a defined measurement solution.

11. A method as claimed in one of the preceding claims, where the second biopolymer (2) is bonded by means of one end to the surface of the first electrode (E1) via a covalent bond or via a linker.

12. A method as claimed in claim 11, where the first electrode (E1) is made of one of the following materials: plastic, ceramic, glass or metal.

13. A method as claimed in one of the preceding claims, where the first biopolymer (1) is a single-stranded DNA or RNA which is complementary to the second biopolymer (2).

14. A method as claimed in claim 13, where in step b), the change in voltage and/or current caused by hybridization of the biopolymers (1, 2) is measured.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

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Zur Erklärung der Zweibuchstaben-Codes, und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(54) Title: METHOD FOR DETECTING AND QUANTIFYING FIRST BIOPOLYMERS THAT ARE LOCATED IN A LIQUID

(54) Bezeichnung: VERFAHREN ZUM NACHWEIS UND ZUR QUANTIFIZIERUNG VON IN EINER FLUSSIGKEIT BEFINDLICHEN ERSTEN BIOPOLYMEREN

(57) Abstract: The invention relates to a method for detecting and quantifying first biopolymers (1) that are located in a liquid. Second biopolymers (2) having a specific affinity towards the first biopolymers (1) to be detected are bound to the surface of a first electrode (E1). The first and at least one second electrode are dipped into the liquid. The inventive method comprises the following steps: a) applying a changeable voltage and/or a changeable current over the first (E1) and second electrode (E2) and b) measuring the direct change of voltage and/or current, whereby said change is the result of the accumulation of the first biomolecules (1) to the second biomolecules (2).

(57) Zusammenfassung: Die Erfindung betrifft ein Verfahren zum Nachweis und zur Quantifizierung von in einer Flüssigkeit befindlichen ersten Biopolymeren (1), wobei zweite zu den nachzuweisenden ersten Biopolymeren (1) eine spezifische Affinität besitzende Biopolymere (2) an die Oberfläche einer ersten Elektrode (E1) gebunden sind und wobei die erste und mindestens eine zweite Elektrode in die Flüssigkeit eingetaucht sind, mit folgenden Schritten: a) Anlegen einer veränderlichen Spannung und/oder eines veränderlichen Stroms über der ersten (E1) und der zweiten Elektrode (E2) und b) Messung einer durch die Anlagerung der ersten Biomoleküle (1) an die zweiten Biomoleküle (2) bedingten unmittelbaren Änderung der Spannung und/oder des Stroms.

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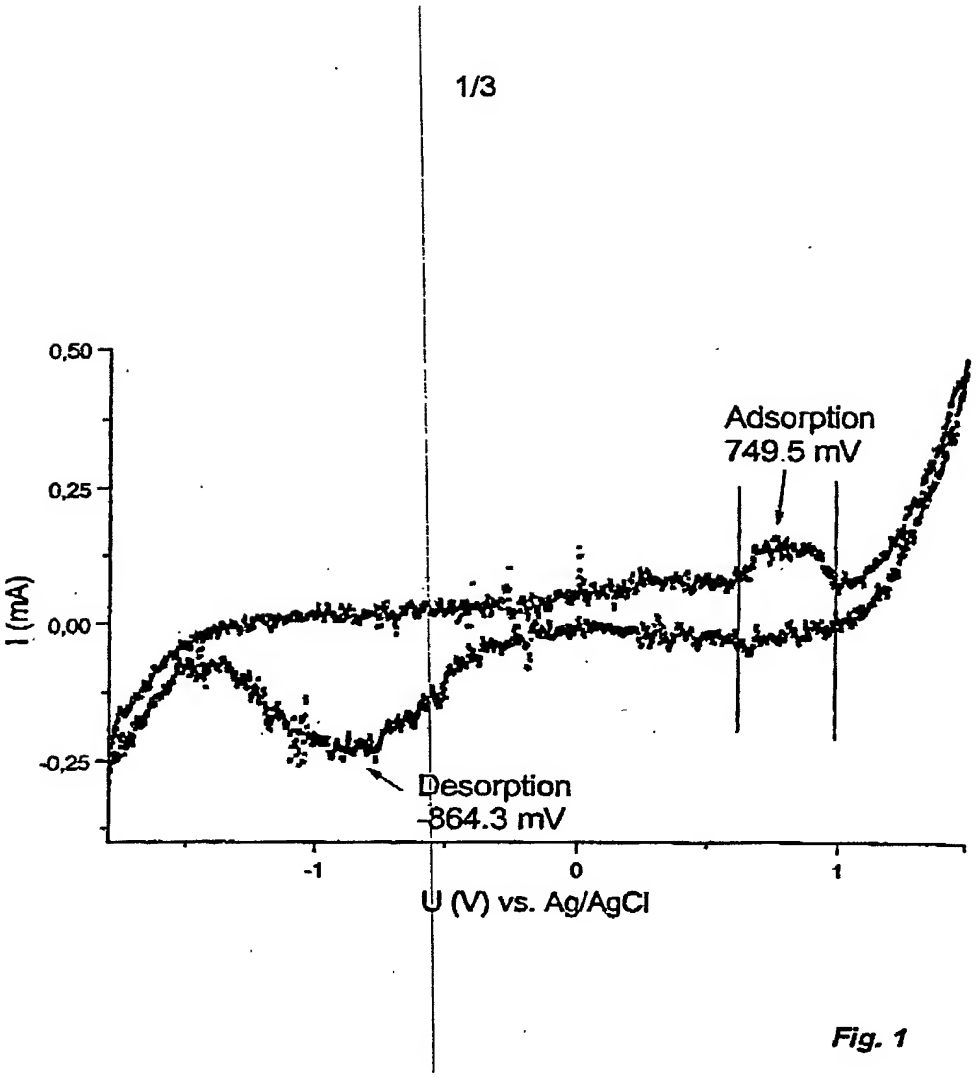
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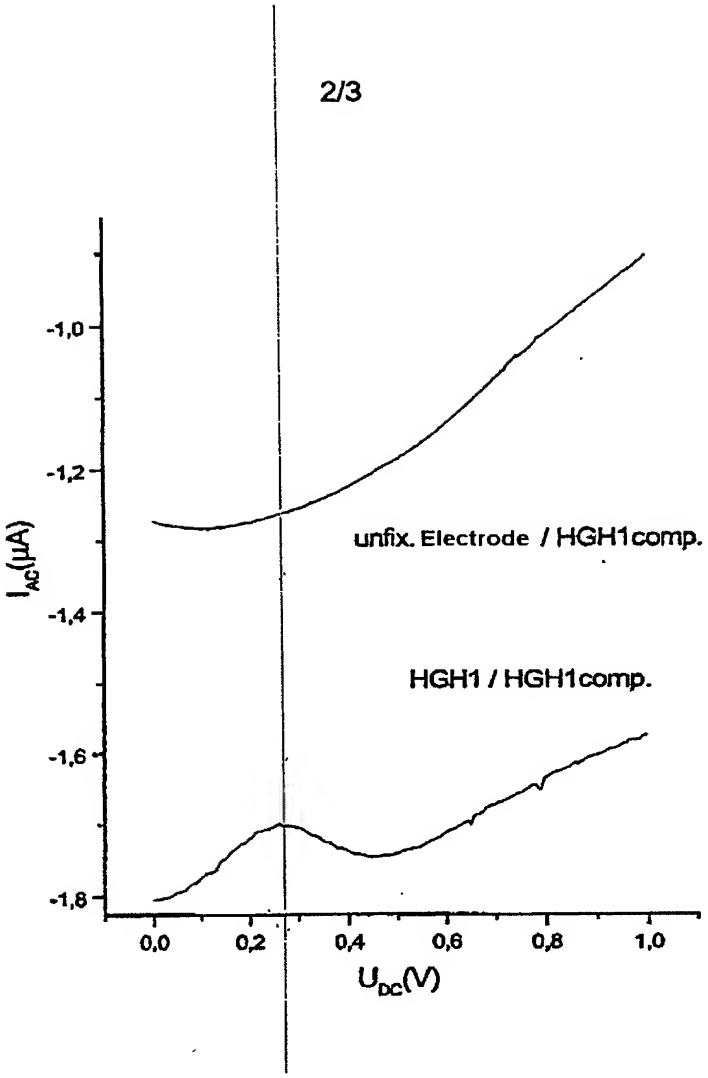
Fig. 1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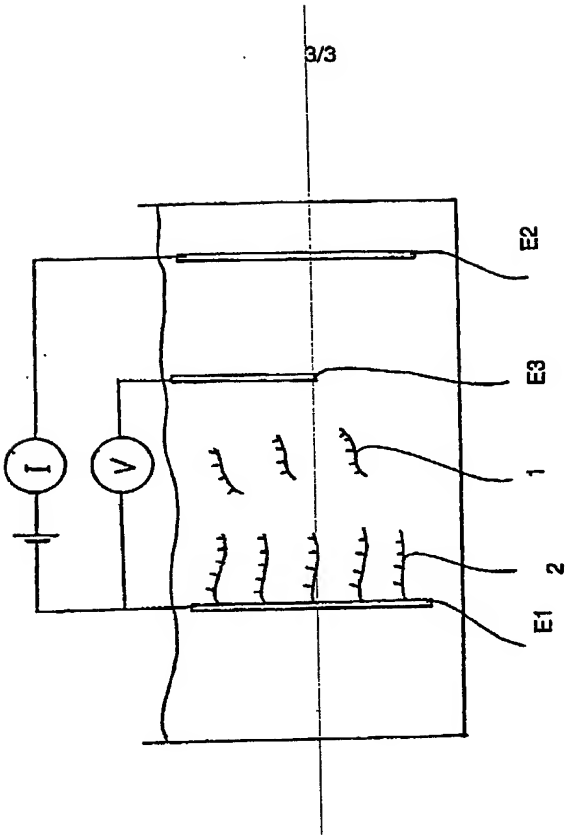

Fig. 3

SEQUENCE LISTING

<110> november Aktiengesellschaft
Gesellschaft für Molekulare Medizin

<120> Method for detecting and quantifying biopolymers that are located in a liquid

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10069840 .062602

Attorney's Docket No.: 10848-021US1
Client's Ref. No.: 422073GA-go

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled *Method For Detecting and Quantifying First Biopolymers That Are Located in a Liquid*, the specification of which:

[] is attached hereto.
[X] was filed on February 26, 2002 as Application Serial No. 10/069,840.
[] was described and claimed in PCT International Application No. ________________ filed on ________________ and as amended under PCT Article 19 on ________________.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Application No.	Filing Date	Priority Claimed
PCT	PCT/DE00/02757	August 12, 2000	[X] Yes [] No
Germany	199 40 647.2	August 26, 1999	[X] Yes [] No

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

Combined Declaration and Power of Attorney

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